Effect of Temperature, Exposure Interval, and Depth of Diatomaceous Earth Treatment on Distribution, Mortality, and Progeny Production of Lesser Grain Borer (Coleoptera: Bostrichidae) in Stored Wheat

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ABSTRACT Diatomaceous earth (DE) can be used as a surface treatment in stored wheat *Triticum aestivum* (L.) to control pest infestations. However, it is not known how the thickness of the DE-treated wheat layer or grain temperature impact effectiveness. Therefore, we conducted an experiment in growth chambers to assess the effect of different surface layers of hard winter wheat combined with DE on spatial distribution, adult survival, and progeny production of lesser grain borer, *Rhyzopertha dominica* (F.), and to determine whether temperature and exposure interval modified this effect. When adult lesser grain borers were released in experimental towers containing untreated wheat or wheat admixed with DE to a surface layer depth of 15.2, 22.9, or 30.5 cm, they were able to penetrate all DE layers and oviposit in the untreated wheat below. However, survival was significantly reduced in adults exposed to DE. Survival decreased both with increasing depth of the DE-treated wheat and with exposure interval. Temperature had no effect on adult survival, but significantly more progeny were produced at 32 than at 27°C. Progeny production was inversely correlated with the depth of the DE-treated layer. Vertical distribution patterns of parental beetles were not significantly different among treatments or exposure intervals; however, more insects were found at greater depths at 32 than at 27°C. The F_1 production was reduced by 22% at the thickest DE-treated layer. However, we conclude that this level of survival could leave a residual population of lesser grain borers that would probably be above an allowable threshold for insect damage.

KEY WORDS *Rhyzopertha dominica*, diatomaceous earth, wheat, control, movement

Lesser grain borer, *Rhyzopertha dominica* (F.), is a serious pest of stored raw commodities worldwide and most commonly infests grain after it is harvested and stored (Potter 1935). Initial infestation of the grain mass generally occurs at the surface and then spreads downward (Sharangapani and Pingale 1957, Sutrees 1965, Keever 1983, Hagstrum et al. 1994, Vela-Coiffier et al. 1997, Hagstrum 2001). Control of lesser grain borer is difficult because most of the life cycle is spent inside the grain kernel. Eggs are laid externally on the grain kernels, and the first instar chews its way inside where it feeds and develops. Upon reaching the adult stage, the insect bores out of the kernel, creating a large exit hole (Potter 1935). This exit hole is often indicative of an insect-damaged kernel (IDK) which is often a factor in the grading and marketing of grain (Flinn et al. 2004).

In the United States, there are few commercial insecticides registered to control the lesser grain borer. Historically, malathion was used as a grain protectant, but numerous studies have shown resistance to this insecticide (e.g., Subramanyam and Hagstrum 1996). Chlorpyrifos-methyl (Reldan) is labeled for direct application to stored wheat *Triticum aestivum* (L.), but the lesser grain borer is not listed on the product label. Studies have shown resistance of the lesser grain borer to chlorpyrifos-methyl (Zettler and Cuperus 1990, Arthur 1992, Guedes et al. 1996), however, this particular formation containing chlorpyrifos-methyl has been withdrawn from the market in the United States. The insect growth regulator (IGR) methoprene can affect larval development if the egg is contaminated with the insecticide residue (Samson et al. 1990, Daglish and Pulvirenti 1998, Arthur 2004), but IGRs do not affect the immature stages of lesser grain borers that are within the grain kernel. In addition, they generally do not kill adults, which is the only other life stage likely to be exposed.

New formulations of diatomaceous earths (DE), a reduced risk insecticide, are being developed to suppress insects in stored grain. Diatomaceous earth is a natural product mined from deposits of fossilized diatoms (Quarles 1992, Golob 1997). The dust interferes with water transpiration by absorption of cuticular lipids and causes desiccation (Korunic 1998,

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Subramanyam and Roesli 2000). Some research reports show that the lesser grain borer is one of the most difficult stored product insects to kill with DE (Quarles 1992, Fields and Korunic 2000, Subramanyam and Roesli 2000). Other more recent studies indicate that it can be less susceptible than the red flour beetle, *Tribolium castaneum* (Herbst), to new European commercial DE products (Athanassiou and Kavallieratos 2005, Kavallieratos et al. 2005). However, because the lesser grain borer produces a true IDK, it is probably a more important economic pest of grain in the United States than the red flour beetle. Environmental conditions impact DE efficacy, which generally decreases with increases in grain moisture content or relative humidity. However, interspecific variation has been reported in how temperature influences efficacy (Fields and Korunic 2000)

A disadvantage to using DE in bulk grain is that labeled rates may negatively affect physical properties of grain, such as flow rate and test weight (Korunic et al. 1996, 1998). One way to alleviate this problem is by using it as a surface treatment, i.e., as a mixture of DE and wheat added to the top of a grain mass. Diatomaceous earth is thought to attach to the cuticle of the lesser grain borers as they disperse downward from the grain surface through the treated layer, and into the grain mass. If the beetles do not obtain a lethal dose while passing through the treated layer, then this approach may not be effective. Therefore, the depth of the treated layer is a critical factor. Variation in temperature can influence the rate at which beetles move through grain (Flinn and Hagstrum 1998); in turn, this could influence the exposure of beetles to the DE-treated layer.

There are relatively few published reports regarding efficacy of DE as a surface treatment in stored wheat (Nickson et al. 1994, Subramanyam et al. 1994). Therefore, the experimental objectives were to 1) evaluate the effects of temperature, amount of time the insects are exposed to the DE-treated layer, and treated layer depth on adult survival; 2) determine to what extent lesser grain borer adults disperse through different depths of grain treated with DE; and 3) determine progeny production and F_1 adult survival when lesser grain borers are exposed to different combinations of DE-treated layer depth and temperature.

Materials and Methods

The experiment was conducted in two walk-in environmental growth chambers measuring 3.7 by 1.83 by 1.83 m, and containing metal shelving on the side walls. Both chambers were equipped with environmental controls for temperature and relative humidity. The temperature in one chamber was set to 27°C, and the other chamber was set to 32°C. Relative humidity in each chamber was maintained at ≈50% with a range of 45.5–64.4%. Environmental conditions in the chambers were monitored using a HOBO recording sensor (Onset Computers, Bourne, MA).

Experimental units were constructed using 7.6- (8.9-) cm inside (outside) diameter polyvinyl chloride (PVC) pipe. Individual rings measuring 7.6 cm in height were cut from the pipe, and 12 of these rings were taped together with duct tape to form a vertical column (hereafter referred to as "tower"), measuring 91.2 cm. Twenty-four towers were constructed, and each tower was closed at the bottom with a flat PVC hub and closed at the top with a PVC cap. A 1.27-cm-diameter hole was drilled into the top ring of the tower. Three temperature cables attached to a HOBO sensor were inserted through the holes. One cable was placed at the bottom of the tower, a second cable was placed in the middle of the tower, and the third cable was placed so that it would rest on top of the wheat once the tower was filled. This was done to make sure that the temperature throughout the entire tower was consistent. Actual temperatures (mean ± SEM) in the towers of wheat for all repetitions were 27.4 ± 0.1 and 31.9 ± 0.2 in the 27 and 32°C chamber, respectively.

Twelve towers were placed in each of the two temperature chambers. Three towers per chamber served as controls (no DE treatment). These towers were filled with ≈2.6 kg hard red winter wheat, which extended to the top of the third ring, leaving a head space of 15.2 cm. The remaining nine towers contained wheat, a portion of which (the surface layer) was treated with the DE product Protect-It (Hedley Technologies, Mississauga, Ontario, Canada) at the labeled rate of 400 ppm (milligrams of product per kilogram of wheat). Three towers received each of three DE treatments. Treatments consisted of the top 15.2, 22.9, or 30.5 cm of the wheat having DE incorporated into it as an admixture. To achieve this, towers filled first with untreated wheat to the top of the fifth, sixth, or seventh ring and then filled with 30.5, 22.9, or 15.2 cm of DE surface-treated wheat, respectively.

Each 7.6-cm ring can hold 261 g of wheat. Therefore, to attain the labeled rate for DE, batches of 522, 783, and 1,044 g of wheat, corresponding to the 15.2-, 22.9-, and 30.5-cm surface treatments, respectively, were weighed out. The DE was then weighed according to the labeled rate and mixed with wheat by placing the wheat in a 3.8-liter glass mason jar, adding the DE, and hand-shaking and rotating the jar for 1 min. One hundred 1- to 2-wk old adult lesser grain borers (≈1:1 female:male ratio) were obtained from pesticide-susceptible colonies reared at 30°C, 60% RH, on Karl variety hard red winter wheat, no light:dark cycle. These adults were collected from the stock wheat cultures by sieving the wheat through a standard #12 sieves and collecting the adults in a pan below the sieve. Voucher specimens of lesser grain borers were deposited in the Kansas State University Museum of Entomological Prairie Arthropod Research under Lot No. 162. The lesser grain borers were placed on the top surface of the wheat in each tower, and then the tower was closed with a PVC cap. Insects were exposed for 7, 10, or 14 d in each treatment (untreated wheat and the three surface layer depths of DE) for a total of 12 treatment combinations at each temperature. The experiment was a split-split-plot design. The whole plot factor was temperature, the whole plot unit was the chamber, the sub-plot factor was exposure interval, the sub-plot unit was a set of four towers, the sub-sub-plot factor was the depth of the DE-treated layer, and the sub-sub-plot unit was each tower. Each replicate consisted of four treatments (control plus three depths of DE), two temperatures, and three exposure intervals, for a total of 24 towers. Five replicates of the experiment were done from May 2002 through December 2003.

After each exposure interval, the top two rings and the HOBO computer and cables were removed from the tower. Sequentially, the duct tape holding the rings together was removed, a metal plate (14.0 by 9.5 cm) was slid between the adjoining rings, and then the top ring and plate were lifted off the cylinder and wheat from that ring transferred to a 177.4-ml clear plastic container (Consolidated Plastics Company, Inc., Twinsburg, OH). Each container had a 2.5-cm-diameter hole in the side, which was covered with a round copper mesh screen (3 cm in diameter) to allow for airflow.

Survival and vertical distribution of parental adults were assessed by sifting the wheat from each container, counting and tabulating the number of live and dead adult lesser grain borers, and removing all parent adults. The wheat then was placed back into the containers and held for 8 wk under the original temperature conditions. This period was long enough to allow for the F_1 progeny to develop to adults at both temperatures. These adults were then sieved from each container, and live and dead insects were counted.

Data were analyzed using the Proc Mixed or General Linear model (GLM) SAS procedure (SAS Institute 2002). Raw data were transformed by arcsine square root, and statistical analyses were performed on the transformed data. Treatment means for the various statistical tests were separated using the Waller–Duncan k-ratio t-test.

Total adult survival was compared across all DE-layer treatments, temperatures, and exposure intervals. A covariance model was used to analyze the distribution of insects among the 10 rings (strata) in each tower. To assess the average vertical displacement of beetles from the release point at the top of the wheat mass among DE treatments, temperatures, and exposure intervals, each stratum was assigned a number from 1 to 10, with a 1 representing the uppermost stratum. These numbers were multiplied by the number of live insects in the same stratum and then summed over all strata. This sum was then divided by the total number of live insects in each tower to obtain a relative index of net displacement (vertical distribution).

To compare the proportion of insects within and below the DE-treated layers at each temperature and exposure interval, the numbers of live, dead, and total lesser grain borers in each stratum in the DE-treated layer was divided by 15.2, 22.9, or 30.5 cm, depending on the DE treatment, to determine a uniform (per stratum) number within the DE-treated layer. Below the treated layer, the number of lesser grain borers in each stratum was divided by the number of centimeters corresponding to the remaining portion of untreated wheat.

Table 1. Mean ± SEM percentage of survival of P_1 lesser grain borer adults when exposed for different durations to DE-treated and untreated wheat for each exposure interval

Treatment (DE layer depth, cm)	Exposure interval (d)		
	7	10	14
Untreated control	99.8 ± 0.2a	99.6 ± 0.4a	99.7 ± 0.2a
15.2	66.0 ± 5.5b	56.5 ± 7.0b	49.1 ± 7.7b
22.9	37.2 ± 5.3c	28.8 ± 6.6c	26.2 ± 6.0c
30.5	26.9 ± 4.3c	18.2 ± 3.4c	14.8 ± 3.2c

Means within columns followed by different letters are significantly different ($P < 0.05$; Waller–Duncan k-ratio t-test; SAS Institute 2002).

When the actual value of lesser grain borers within and below the DE layers was transformed to a proportional value and converted to a percentage, the populations of live, and dead lesser grain borers were analyzed for differences between the DE layers and the untreated wheat below. These percentages were the values used for statistical analysis. Progeny production and percent survival of progeny were compared across all DE treatments, temperatures, and exposure intervals. To determine whether population growth was affected by DE-treatments, a proportion was obtained by dividing the number of progeny from each tower by the initial number of 100 lesser grain borer adults. The distributions of progeny produced were also compared as percentages within and below the DE treatments, between temperatures, and among exposure intervals.

Results

Survival. Differences in total percentage of adult survival of lesser grain borers throughout all strata within each tower were significant for exposure interval and DE treatment, but not for temperature ($F = 6.9$; df = 2, 16; $P < 0.01$; $F = 240.6$; df = 3, 72; $P < 0.01$; and $F = 3.03$; df = 1, 4; $P = 0.16$, respectively). Survival in DE treatments was significantly lower than in the untreated control, and it declined with increasing depth of the DE layer (Table 1). Survival also declined with increasing exposure interval, but the differences were not significant between the 10- and 14-d exposure interval (Table 1).

Distribution. The vertical distribution of live adult lesser grain borers was significantly affected by DE treatments ($F = 67.8$; df = 3, 936; $P < 0.01$). Within treatments, distribution also differed among strata ($F = 8.2$; df = 9, 936; $P < 0.01$). However, distribution was not affected by temperature ($F = 2.8$; df = 1, 4; $P = 0.17$) or exposure interval ($F = 2.3$; df = 2, 16; $P = 0.13$). There was a significant interaction between strata and temperature, strata and exposure interval, and strata and DE treatment ($P < 0.01$). Figure 1 shows the mean number of live lesser grain borer adults in each stratum for the individual DE treatments, and for untreated wheat, with data combined across temperatures and exposure intervals. There was no consistent pattern in the dispersion of lesser grain

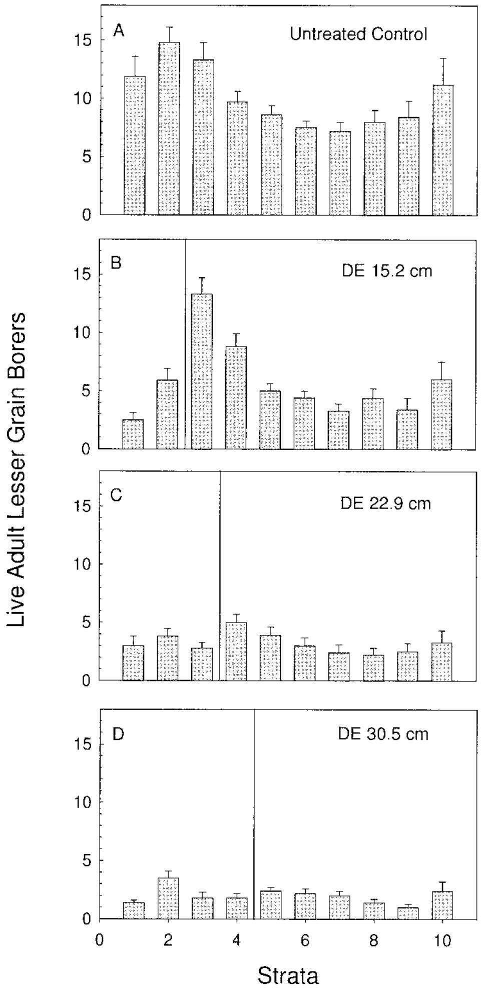

Fig. 1. (A–D) Number of live adult lesser grain borers in each stratum in untreated wheat and in wheat with three treatments of DE-treated wheat layered on top of untreated wheat. Data from different exposure intervals and temperatures were combined. The vertical lines in B–D show the number of strata below the DE-treated layer.

borers among DE treatments or between DE treatments and the untreated control.

Likewise, there was no difference among DE treatments in the vertical displacement (mean distance traveled) of live lesser grain borers at either 27 or 32°C ($F = 1.5$; df = 3, 68; $P = 0.24$; and $F = 0.4$, df = 3, $P = 0.75$, respectively). However, when live and dead adults were combined, there were significant differences among treatments in the vertical displacement of lesser grain borers at each temperature (Table 2). More insects were found at greater depths at 32 than at 27°C, but there was no significant difference in mean vertical displacement of live and dead lesser grain borers between DE-treated and untreated wheat.

The percentage of live adults within versus below the DE layer was significantly influenced by DE treatment depth and temperature ($F = 39.7$; df = 1, 68; $P < 0.01$; and $F = 8.4$; df = 2, 68; $P < 0.01$, respectively); but exposure interval had no effect ($F = 1.1$; df = 2, 68; $P = 0.34$). At both temperatures, as the depth of the DE layer increased, there was a general trend for an increase in the percentage of live beetles within the DE layer and a decreasing percentage below the DE layer. However, most of differences among treatments were not significant (Table 3). At 27°C, there was a significantly larger percentage of live beetles at the 22.9- and 30.5-cm depths than at the 15.2-cm depth, whereas at 32°C, the percentages among treatments were more similar (Table 3). At 32°C, the percentage of live beetles below versus above the DE layer was greater than at 27°C.

The percentage of dead adults below versus above the DE layer was significantly affected by the DE treatment ($F = 4.4$; df = 2, 48; $P = 0.02$), but not by temperature or exposure interval ($F = 3.83$; df = 1, 4; $P = 0.12$; and $F = 0.02$; df = 2, 16; $P = 0.99$). The percentages of dead adult lesser grain borers were significantly higher within the DE-treated layer at all three depths than in the untreated wheat below ($P < 0.01$). The percentage of dead lesser grain borers within the DE layer was highest at 30.5 cm (Table 4).

Progeny Production. Total production of F_1 adults was significantly affected by the DE treatment ($F = 55.4$; df = 2, 16; $P < 0.01$) and by the exposure interval ($F = 13.0$; df = 2, 16; $P < 0.01$), but not by temperature ($F = 4.6$; df = 1, 4; $P = 0.10$). There were also significant interactions between temperature and treatment ($F = 9.1$; df = 3, 72; $P < 0.01$) and temperature and exposure interval ($F = 7.3$; df = 2, 16; $P < 0.01$).

Table 2. Mean ± SEM vertical displacement of combined live and dead P_1 lesser grain borer adults at different depths of DE-treated wheat and in untreated wheat at each temperature

Treatment (DE layer depth, cm)	27°C	32°C
Untreated control	4.6 ± 0.4a	5.9 ± 0.4a
15.2	3.6 ± 0.4ab	4.5 ± 0.4ab
22.9	3.1 ± 0.3b	4.5 ± 0.4b
30.5	3.4 ± 0.2b	4.2 ± 0.3b

Means within columns followed by different letters are significantly different ($P < 0.05$; Waller–Duncan *k*-ratio *t*-test; SAS Institute 2002).

Table 3. Effect of depth of the DE-treated layer and temperature on mean ± SEM percentage of live P_1 lesser grain borer adults found within and below the DE-treated layer

Treatment (DE layer depth, cm)	% live lesser grain borers[a]			
	27°C		32°C	
	within DE	below DE	within DE	below DE
15.2	44.5 ± 6.5	55.5 ± 6.5a	28.4 ± 6.2	71.6 ± 6.2a
22.9	66.7 ± 7.7	33.4 ± 7.7b	35.6 ± 6.0	66.4 ± 5.9a
30.5	69.2 ± 6.5	30.8 ± 6.5b	41.6 ± 4.4	58.4 ± 4.4a

Means within columns followed by different letters are significantly different ($P < 0.05$; Waller–Duncan *k*-ratio *t*-test; SAS Institute 2002).

[a] Percentages shown were converted to a per stratum basis to adjust for differences in proportions of DE-treated versus -untreated wheat among treatments.

Table 4. Effect of depth of the DE-treated layer on the mean ± SEM percentage of dead P_1 lesser grain borer adults within and below the DE-treated layer

Treatment (DE layer depth, cm)	% dead lesser grain borers[a]	
	within DE	below DE
15.2	75.9 ± 2.8ab	24.1 ± 2.8ab
22.9	75.0 ± 2.7b	25.0 ± 2.7a
30.5	83.4 ± 2.6a	16.6 ± 2.6b

Means within columns followed by different letters are significantly different ($P < 0.05$; Waller–Duncan k-ratio t-test; SAS Institute 2002).
[a] Percentages shown were converted to a per stratum basis to adjust for differences in proportions of DE-treated versus -untreated wheat among treatments..

Table 6. Mean ± SEM population growth rate between the P_1 and F_1 generations of adult lesser grain borers after release in stored wheat treated with different depths of DE or on untreated wheat at two temperatures and after exposure for either 7, 10, or 14 d

Treatment (DE layer depth, cm)	Exposure interval (d)		
	7	10	14
Untreated	32.1 ± 11.0a	47.9 ± 14.0a	62.2 ± 14.4a
15.2	17.3 ± 9.4ab	29.2 ± 13.9ab	37.0 ± 15.3ab
22.9	8.3 ± 3.9ab	17.1 ± 9.4ab	21.5 ± 10.4ab
30.5	5.2 ± 2.7b	6.2 ± 4.0b	18.3 ± 11.1b

Means within columns for each exposure interval followed by different letters are significantly different ($P < 0.05$; Waller–Duncan k-ratio t-test; SAS Institute 2002).

The number of progeny produced increased as exposure interval increased for all DE treatments and for the untreated control. Progeny production decreased with increasing depth of the DE-treated layer, and the most progeny were produced in the untreated control (Table 5).

The population growth rate, as measured by the difference in number of progeny relative to the original number of parental lesser grain borers, was significantly affected by DE treatment and exposure interval ($F = 49.3$; df = 3, 72; $P < 0.01$; and $F = 13.4$; df = 2, 16; $P < 0.01$, respectively) but not temperature. ($F = 4.6$; df = 1, 4; $P = 0.10$). There were significant interactions between temperature and exposure interval, and temperature and treatment ($F = 7.6$; df = 2, 16; $P < 0.01$; and $F = 9.5$; df = 3, 72; $P < 0.01$, respectively). The greatest population growth rate was in the untreated control for all exposure intervals. The lowest population growth rates were observed for the deepest DE layer (Table 6). As depth increased, population growth rates decreased. There seemed to be a trend for increasing population growth rate as exposure interval increased (Table 6).

Adult Progeny Survival. The percentage survival for F_1 lesser grain borer adults was significantly influenced by DE treatment and temperature ($F = 43.2$; df = 3, 87.1; $P < 0.01$; and $F = 11.2$; df = 1, 4; $P = 0.03$, respectively). However, exposure interval did not affect survival ($F = 0.8$; df = 2, 87.1; $P = 0.46$). There was a significant temperature by treatment interaction ($F = 8.9$; df = 3, 87.1; $P < 0.01$). At both temperatures, there was a trend for decreasing survival with an increase in the depth of the DE-treated layer. The differences among treatments were all significant at 27°C. However, at 32°C the percentage of survival was significantly lower only at 30.5 cm (Table 7).

Distribution of Progeny. The percentage of live adult F_1 lesser grain borers below the DE-treated layer was significantly influenced by the DE treatment and by temperature ($F = 0.8$; df = 2, 47.6; $P < 0.01$; and $F = 8.3$; df = 1, 20.2; $P < 0.01$, respectively), but not exposure interval ($F = 0.9$; df = 2, 20.2; $P = 0.44$). At 27°C, a significantly higher percentage of live F_1 adults was found below the DE-treated layer at 15.2 and 22.9 cm than at 30.5 cm. At 32°C, there was a similar trend for increases in survival below the DE-treated layers. However, there were no significant differences among treatments (Table 7). Within the DE treatments, there was generally a significant difference in the percentage of live beetles found between the DE-treated layer and the wheat below the DE-treated layer ($P < 0.01$). At 27°C, the percentage of live beetles was greater below the DE-treated layers at all depths. At 32°C, the same trend was observed. Above the DE-treated layer, percentage survival at 27°C was significantly lower at 15.2 cm, whereas at 32°C there were no significant differences among DE-layer treatments.

The percentages of dead F_1 adult lesser grain borers below the DE-treated layers were not significantly affected by DE treatment, temperature, or exposure interval ($P > 0.05$). However, nearly all of the dead F_1 adults were distributed within the DE-treated layer, regardless of the depth of the DE treatment (Table 7).

Discussion

There are several published reports documenting the movement of lesser grain borers through wheat (Sharangpani and Pingale 1957; Sutrees 1964, 1965; Keever 1983). However, these reports do not include data regarding movement through a DE-treated sur-

Table 5. Effect of depth of the DE-treated layer and exposure interval on mean ± SEM number of lesser grain borer F_1 adults produced

Treatment (DE layer depth, cm)	Exposure interval (d)		
	7	10	14
Untreated control	3,305.7 ± 1,146.8a	4,766.2 ± 1,359.4a	6,259.4 ± 1,123.2a
15.2	1,702.3 ± 903.8ab	2,769.5 ± 1,261.4ab	3,713.5 ± 1,530.9ab
22.9	1,017.0 ± 553.7ab	1,706.9 ± 936.6ab	2,265.6 ± 1,123.7ab
30.5	502.0 ± 271.4b	683.3 ± 453.9b	1,814.8 ± 1,118.3b

Means within columns followed by different letters are significantly different ($P < 0.05$; Waller–Duncan k-ratio t-test; SAS Institute 2002).

Table 7. Effect of depth of DE-treated wheat at each of two temperatures on the mean ± SEM percentage occurrence of F_1 lesser grain borer adults within and below the DE-treated layer and on percentage of survival

DE layer depth, cm	% live lesser grain borer		% dead lesser grain borer		% total survival
	within DE	below DE	within DE	below DE	
27°C					
15.2	13.5 ± 4.7b	86.5 ± 4.7a	100.0 ± 0a	0.0 ± 0.0a	91.8 ± 1.4a
22.9	33.9 ± 10.1ab	66.1 ± 10.1ab	100.0 ± 0a	0.0 ± 0.0a	59.9 ± 6.7b
30.5	41.5 ± 9.3a	58.5 ± 9.3b	99.3 ± 0.5a	0.7 ± 0.5a	39.4 ± 7.7c
32°C					
15.2	14.8 ± 5.0a	85.2 ± 5.0a	97.2 ± 1.9a	2.8 ± 1.9a	96.8 ± 1.0a
22.9	10.9 ± 3.7a	89.1 ± 3.7a	100.0 ± 0a	0.0 ± 0.0a	90.5 ± 4.6a
30.5	18.8 ± 5.3a	81.2 ± 5.3a	99.6 ± 0.3a	0.3 ± 0.3a	74.7 ± 6.4b

Means for percentage of live and dead lesser grain borer within the same column for each temp followed by different letters are significantly different ($P < 0.05$; Waller–Duncan *k*-ratio *t*-test; SAS Institute 2002).

face layer. These behavioral studies report that movement of the lesser grain borer is usually a slow downward process that occurs mainly in the center of the grain mass. These findings are supported by the current study as well, in that adult lesser grain borers were vertically distributed throughout most of the strata within the 7- to 14-d exposure period. In addition, lesser grain borers have been shown to increase their activity and distribution at higher temperatures. Sutrees (1965) showed an 80% increase in distribution of lesser grain borers in grain when temperatures increased from 15 to 35°C. This finding agrees with our study in which lesser grain borers had a wider distribution at 32°C than at 27°C, suggesting an increased rate of dispersal.

Hagstrum et al. (1994) reported that insect pests of stored grain often enter a grain bin environment through the headspace of the bin and concentrate in the center of the grain mass. Therefore, using DE as a surface layer treatment might protect the area of infestation as well as reduce some of the problems associated with the use of DE on an entire grain mass. The results of this study showed that the DE treatments did not prevent lesser grain borers from penetrating into the untreated wheat. However, the greater the depth of the DE treatment, the lower the population of lesser grain borers. Although lesser grain borers are sometimes difficult to kill with DE, Fields and Korunic (2000) documented a 90% mortality rate on wheat treated with 300 ppm of Protect-It after a 14-d exposure at 25°C and a grain moisture content of 11.8%. Our results for Protect-It at 400 ppm at the greatest DE depth, and after a 14-d exposure, were similar with respect to mortality (≈85%), to that observed by Fields and Korunic (2000).

Mortality of parental lesser grain borers was directly related both to the depth of the DE-treated layer and exposure interval. Several other studies have shown a positive relationship between time exposed to DE and mortality for other species of stored-product beetles (Vrba et al. 1983, White and Loschiavo 1989, McLaughlin 1994, Arthur 2000). Vrba et al. (1983) exposed confused flour beetles, *Tribolium confusum* (DuVal) for 1, 2, 3, or 5 h to the DE product silica aerogel (Aerosil 380) and demonstrated an increase in mortality with increased exposure interval. Arthur (2000) conducted tests in which red flour beetles and confused flour beetles were exposed for various time intervals to a DE product (Protect-It at 5 g/m^2), after which mortality was assessed immediately after exposure and then 1 wk later. Both initial mortality and final mortality were directly related to exposure interval to DE. More recent studies with DE also show that short exposure intervals of 1- to 2-d exposure interval may not give satisfactory control of lesser grain borer and other stored-grain species, and longer exposure intervals are necessary for complete mortality (Athanassiou et al. 2004, 2005).

Efficacy of DE has been shown to be reduced when the insects were allowed access to food. McLaughlin (1994) exposed granary weevils, *Sitophilus granarius* (L.), for 21 d on a dust–wheat mixture and also on a treated surface. On both substrates, time of exposure was directly related to mortality, but mortality was reduced when food was provided. In our study, the insects had a readily available food source, and the freedom to move into or out of the treated environment, yet exposure interval was still significant for initial mortality. However, the depth of the DE-treated layer had a much greater effect on lesser grain borer mortality compared with exposure interval. In addition, the relatively higher percentage of dead borers within the DE layer compared with the live borers suggests that dying beetles may have remained to a greater degree in the upper (treated) strata than those that survived exposure to the DE.

Higher temperatures generally lead to greater mortality of stored-product beetles when exposed on grain or surface substrates (Quarles and Winn 1996, Korunic 1998, Arthur 2000, Fields and Korunic 2000). For the lesser grain borer, Aldryhim (1993) reported that mortality on different wheat classes treated with the DE product Dryacide increased as temperature increased. However, in our study temperature did not significantly affect mortality of the initial adult lesser grain borers, but it did affect the proportion of progeny produced, with a significantly greater number of progeny produced at 32°C than at 27°C. This suggests that temperature may have had an effect on oviposition, preadult survival, or both. In contrast to other stored-grain beetles, lesser grain borers generally reproduce and develop optimally at higher temperatures (Howe

1950). Hagstrum (1996) predicted population growth rates for the lesser grain borer at different temperatures and grain moisture contents and documented an increase in egg production by 1.47–2.99 times with every 5°C increase in temperature or 2% increase in grain moisture.

Nickson et al. (1994) used Dryacide in combination with cooling (aeration) at three different commercial grain silos and found that the combination of treatments provided effective control against stored-product insects in Australia. Subramanyam et al. (1994) studied the effects of Insecto as a surface treatment for wheat and barley in metal barrels at different concentrations, as well as in the laboratory for six species of stored-product insects, including the lesser grain borer. Suppression of lesser grain borers after 7- or 14-d exposure ranged from 55 to 70% in the barrel studies, whereas 96–100% mortality was obtained in the laboratory. Their results confirm that the lesser grain borer could reproduce when it had access to untreated wheat below a surface layer treated with DE.

Our study demonstrates that the lesser grain borer was capable of penetrating a DE-treated surface layer and reproducing in the untreated wheat. Increasing the depth of the treated layer resulted in greater adult mortality, reduced progeny production, and also decreased population growth rates. These findings suggest that increasing the depth or volume of DE-treated wheat could result in increased efficacy of Protect-It or other diatomaceous earth products. It is unclear whether DE alone, and at rates acceptable in stored grain facilities, can reduce lesser grain borers to tolerable levels. However, using DE in conjunction with aeration or reduced-risk insecticides (Stathers et al. 2002) may increase the breadth of applications of DE products against this pest.

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